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Optimization of anti-infective multiparticulate systems for pulmonary delivery

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List of abbreviations

Abbreviation	Word
A549	Human lung carcinoma cell line
ANOVA	Analysis of variance
ºC	Degree Celsius
DCM	Dichloro methane
DF	Degree of freedom
DMSO	Dimethyl sulfoxide
DPI	Dry powder inhaler
DL%	Drug loading percentage
DLS	Dynamic light scattering
DSC	Differential scanning calorimetry
EE%	Entrapment efficiency percentage
ED%	Emitted dose percentage
f2	Similarity factor
FPF	Fine particle fraction
GRAS	Generally recognized as safe
HIV	Human immune deficiency virus
IC_{50}	50% inhibitory concentration
I%	Percentage inhibition
LDH	Lactate dehydrogenase
LPPs	Large porous particles
MDI	Metred dose inhaler
MIC	Minimum inhibitory concentration
MMAD	Mass median aerodynamic diameter
MN	Miconazole nitrate
MPs	Microparticles
MS	Mean of squares
MTT	3-[4, 5-dimethyl-2-thiazolyl]-2, 5-diphenyl-2H-tetrazolium-bromide
NCMs	Nano composite microparticles
Nm	Nano meter
NPs	Nanoparticles
PBS	Phosphate buffer saline
PDI	Poly dispersity index
PLGA	Poly lactide co-glycolide
PVA	Poly vinyl alcohol
PVR	Organic to aqueous phase volume ratio
RH	Relative humidity
RP	Respirable particle fraction
Rpm	Revolution per minute
SD	Standard deviation
SEM	Scanning electron microscopy
S_f/S_i	Powder re-dispersibility
S_f	Size of dispersed particles
S_i	Size of initial nanoparticles before spray drying

SLN	Solid lipid nanoparticles
SS	Sum of squares
TEM	Transmission electron microscopy
Tg	Glass transition temperature
TGA	Thermogravimetric analysis
TSI	Twin stage impinge
µg	Microgram
µm	Micrometer
VMD	Volume mean diameter
XRPD	x-ray powder diffraction
θ	Angle of repose
ζ	Zeta potential
ρ	Tapped density

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Optimization of anti-infective multiparticulate systems for pulmonary delivery

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Pulmonary fungal infections have increased nowadays due to the increase in the number of immunocompromised patients. These infections are usually treated via systemic route. To minimize the side effects associated with systemic delivery, local targeting to the lung is being exploited as a suitable delivery route. Efficient delivery to the lung requires the fabrication of particles of suitable particle size and properties to ensure their alveolar deposition.

In this thesis, miconazole nitrate (MN) was encapsulated into poly lactide co-glycolide (PLGA) nanoparticles (NPs) followed by their spray drying to obtain nanocomposite microparticles (NCMs) for local treatment of pulmonary fungal infections.

MN is one of the earliest discovered azoles, yet it is still commonly used for treatment of fungal infections due to its diverse mechanisms of actions against the fungal cells.

In chapter I, PLGA NPs of MN were prepared by solvent evaporation method. The effect of formulation variables such as homogenization time, surfactant concentration, drug amount and aqueous phase volume on entrapment efficiency (EE%), drug loading (DL%) and particle size were evaluated. The NPs formulation was further optimized using a full factorial design to study the effect of polymer type (PLGA 75:25 and PLGA 50:50), polymer amount (100, 200 and 300mg) and organic to aqueous phase volume ratio (PVR) (1:2 and 1:4). The obtained particles were characterized in terms of EE%, DL%, particle size and zeta potential (ζ).

The prepared NPs had EE% ranging between 26.12 and 49.58 % and DL% of 3.9 – 9.98%. The size range of the obtained particles was 273.3 - 374.2nm. All the prepared particles had a negative surface charge. Formula F10 prepared with 100 mg PLGA 75:25 and PVR 1:2 had optimum properties, with DL% =9.98% and particle size of 341.9 nm.

In chapter II, the optimized NPs were spray dried to obtain NCMs of suitable size for pulmonary delivery that are able to dissociate into their forming NPs upon contacting lung fluids. Spray drying conditions, namely, inlet temperature and feed concentration were optimized. The effect of different levels of mannitol and leucine

(0, 25 and 50mg) on NCMs properties were then studied according to a full factorial design. The % yield, flowability, mass median aerodynamic diameter (MMAD), powder re-dispersibility (S_f/S_i), moisture and drug content of the obtained NCMs were evaluated. Further studies included the evaluation of: particles' morphology by scanning electron microscopy (SEM), particles' crystalline structure using differential scanning calorimetry (DSC) and x-ray powder diffraction (XRPD), *in vitro* drug release and *in vitro* evaluation of their deposition pattern using the twin stage impinge (TSI).

The spray drying yield of the obtained NCMs was between 23.56 and 52%. The angle of repose (θ) of the obtained particles varied between 38.48 and 48.82°.Their MMAD was in the respirable range (1.14 - 2.26μm). All particles showed good ability to re-disperse into their forming NPs and showed low moisture content varying between 0.22 – 0.68%. S3, S7 and S9 showed optimum characteristics and were subjected to further studies. The obtained particles were spherical in shape with surface texture varying between smooth and corrugated according to the used excipient. The drug was in the amorphous state in all the tested formulae. S7 had the slowest release pattern with only 85% of the drug being released within 2 weeks. On the other hand, S3 had the fastest pattern similar to that obtained by F10. By assessment of their *in vitro* aerosolization properties, the emitted dose (ED%) ranged between 56.64 and 83.3% and fine particle fraction (FPF) between 26.78 and 69%. S7 spray dried with 50 mg leucine had optimum properties; 39.61 % yield, θ = 48.82, MMAD = 1.95μm, superior ability to re-disperse into its forming NPs with S_f/S_i = 1.02, moisture content of 6.3%, corrugated spherical particles with the slowest release pattern and FPF of 69%.

In chapter III, the antifungal activity of the prepared NCMs was assessed by determining their minimum inhibitory concentration (MIC) and comparing it to that of pure MN. Also the cytotoxicity of the prepared particles against lung epithelial cells (A549) was evaluated by MTT assay.

NCMs showed an eight fold higher antifungal activity with MIC = 0.49 μg/ml compared to 3.92 μg/ml obtained by pure MN. The prepared particles were safe to the lung epithelial cells at their MIC.

Keywords: Nanoparticles, poly lactide co-glycolide, solvent evaporation, spray drying, mannitol, leucine, nanocomposite microparticles, pulmonary, miconazole nitrate, fungal infections, cytotoxicity.

GENERAL INTRODUCTION

The respiratory system is responsible for supplying the body with oxygen and removing carbon dioxide. It mainly consists of the airways which are subdivided into two major regions, the upper respiratory tract, extending from the external nares to the larynx. Whereas the larynx, trachea, bronchi, bronchioles, and the lungs constitute the lower respiratory tract **(Figure I).** The lungs contain the alveoli, having a total surface area of $143m^2$ and composed of a single epithelial layer of cells with extracellular matrix surrounded by highly perfused capillaries, they provide maximal surface area for gaseous exchange **(Hakim and Usmani 2014).**

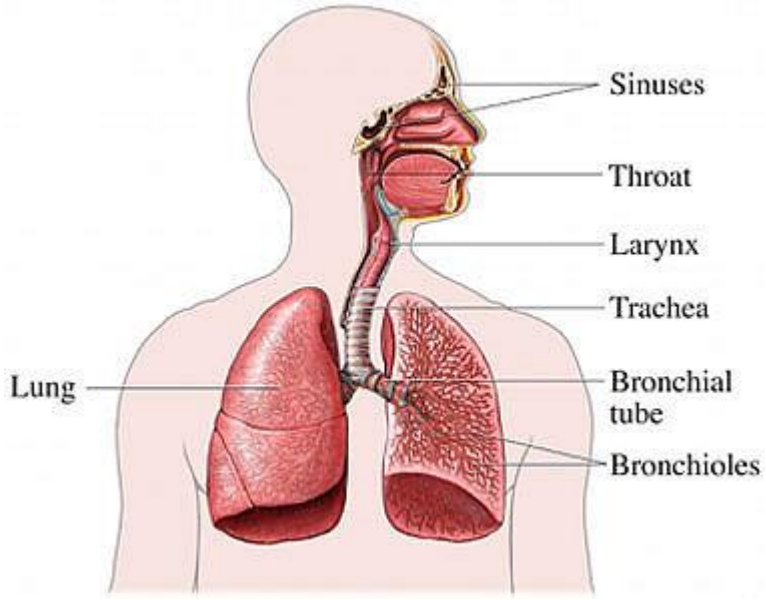

Figure I: Upper and lower respiratory tracts (Hakim and Usmani 2014).

Being a site for gas exchange and in contact with the exterior, the lungs are one of the most susceptible organs to infections due to their exposure to organic, inorganic and biological components that can cause diseases. There are a variety of bacterial, viral, fungal and parasitic infections that infect the lungs and can progress toward systemic infection including pneumonia, TB, influenza and aspergillosis. Infections of the lower respiratory tract are one of the main reasons of morbidity especially in low income countries **(Andrade et al. 2013).**

The increased number of immunocompromised patients in the last few decades associated with human immunodeficiency virus (HIV), cancer, hematologic disorders, and organ transplantations has increased the incidence of pulmonary fungal infections. There are a variety of fungal infections such as histoplasmosis, blastomycosis, coccidioidomycosis, cryptococcosis, aspergillosis, candidiasis and pneumonia that can affect the respiratory tract and cause pulmonary injury with different severities **(Andrade et al. 2013).**

Pulmonary infections are usually treated via the systemic route, either by oral or intravenous administration of antifungal agents such as: amphotericin B, flucytosine and a variety of azole derivatives (fluconazole, itraconazole and miconazole). However, these traditional treatments have poor therapeutic outcomes. Where, some antifungals suffer from slow dissolution as well as erratic and unpredictable bioavailability upon oral administration such as itraconazole which is one of the commonly used azoles. It is effective against some aspergillus infections, mucosal candidal infections, histoplasmosis, blastomycosis, coccidioidomycosis, and other fungal infections and is available either as oral capsules or an oral solution. The oral capsules are well absorbed in acidic media, and so are usually taken with food or acidic beverages to enhance gastric acid secretion and the concurrent use of proton pump inhibitors or antacids should be stopped to avoid problems of variable drug absorption. In contrast to the capsule form, the oral solution requires an empty stomach, and in both cases careful monitoring of the drug level in blood is required **(Limper et al. 2011)**.

Respiratory infections are difficult to treat because microbes usually reside deep in the airways where only a small proportion of the systemically administered drug can access. Consequently, high doses of drugs are required to maintain drug levels above their minimum inhibitory concentrations (MIC) at the infection sites **(Zhou et al. 2015).** Amphotericin B deoxycholate (amphotericin B), the first treatment option for severe fungal infections including aspergillosis, cryptococcosis, candidiasis, and severe cases of histoplasmosis, blastomycosis, coccidioidomycosis, and